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Natural incidence of parasitic bacterium, *Pasteuria* sp. on plant parasitic nematodes in Haryana

MANISH VATS, SATISH MEHTA and R. K. WALIA
Department of Nematology
CCS Haryana Agricultural University
Hisar 25 004, Haryana, India
E-mail: raman31@sancharnet.in

ABSTRACT: **No incidence of *Pasteuria* spp. was recorded (except on *Heterodera cajani*) in field crops infected with *Heterodera avenae, H. zeae, Rotylenchulus reniformis* or citrus infected with *Tylenchulus semipenetrans*. Grape vineyards infected with *Meloidogyne javanica* revealed 56 per cent incidence of *Pasteuria penetrans*. Ten-year-old vineyards showed maximum prevalence of the bacterium. Sugar centrifugal floatation technique was better than modified Baermann's funnel method for the recovery of *Meloidogyne* J2 encumbered with *P. penetrans* spores. Bioassay of soil samples from vineyards proved more useful than routine analysis to estimate the extent of infestation of *P. penetrans*.**

KEY WORDS: Biological control, natural incidence, *Pasteuria penetrans*, plant parasitic nematodes, survey

INTRODUCTION

The mycelial and endospore-forming bacteria (*Pasteuria* spp.) are obligate parasites of nematodes. So far, three species have been reported from among plant parasitic nematodes, *viz.*, *Pasteuria penetrans* parasitic on root-knot nematodes, *P. thornei* on lesion nematodes (Starr and Sayre, 1988), and *P. nishizawae* on cyst nematodes (Sayre *et al.*, 1991). Members of *Pasteuria* group are vastly distributed and have been reported from several countries in association with different groups of nematodes (Chen and Dickson, 1998). A systematic study was conducted to ascertain the natural incidence of *Pasteuria* spp. on economically important phytonematodes of Haryana, and isolation of indigenous strains.

MATERIALS AND METHODS

Random survey

During 1998-99, 96 soil samples were collected from fields infested with *Heterodera avenae* Woll., *H. cajani* Koshy, *H. zeae* Koshy *et al.*, *Rotylenchulus reniformis* Linford & Oliveira, *Tylenchulus semipenetrans* Cobb and *Meloidogyne* spp. from different parts of Haryana. Soil samples were processed by wet screening, followed by modified Baermann's funnel technique (MBFT) (Schindler, 1961). Ten nematodes (infective stages) were picked at random and observed microscopically (400x) for bacterial spore encumbrance. Besides field crops, a few samples from greenhouse cultures of certain populations of *H. cajani*, kitchen garden infested with root-knot nematode and nematode sick-plot harbouring

Heterodera mothi Khan and Husain, were also examined similarly.

Specific survey of root-knot infested grape vineyards

Based on the results obtained through random survey, a pilot study of grape vineyards infested with root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood was conducted during 1999-2000. Eleven samples were processed and examined for the incidence of *P. penetrans* on J2. However, during 2000-01, a more intensive study was conducted. Twenty-five samples were collected from different vineyards around Hisar. From each site, two samples (200cc each) were processed by wet screening; thereafter one sample was further processed by MBFT and the second by sugar centrifugal floatation technique (SCFT) (Caveness and Jensen, 1955).

Bioassay

The remaining soil collected from grape vineyards during 2000-01 was air-dried for about 40 days. After thorough mixing, 20g soil was put in each 5cm Petri-plate. Three such plates were maintained for each site. A 10ml water suspension containing ca. 1000 healthy J2 of *M. javanica* was poured in each Petri-plate. All the plates were stored at 28±1°C in a BOD incubator, after which nematodes were extracted by SCFT and observations were recorded on bacterial spore encumbrance to J2.

RESULTS AND DISCUSSION

Random survey

No incidence of *Pasteuria* was recorded on *H. avenae, H. zeae, H. graminis, R. reniformis* and *T. semipenetrans*. A field population of *H. cajani* (race B) harboured *Pasteuria* infection; however, among eight populations of *H. cajani* maintained in greenhouse, four were infected with *Pasteuria*. Incidence of *Pasteuria* was also recorded on a natural population of *H. mothi* in a root-knot nematode sick-plot (Table 1).

Table 1. Natural incidence of *Pasteuria* species on plant parasitic nematodes (1998-99)

Nematode	Crop/Plant/Source	No. of Samples examined	No. of Samples having *Pasteuria*
Heterodera avenae	Wheat	25	Nil
Heterodera cajani	Cluster bean	3	1
	Kharif pulses	5	Nil
	Different places	8	4
Heterodera graminis	*Cynodon dactylon*	3	Nil
Heterodera mothi	*Cyperus rotundus*	1	1
Heterodera zeae	Maize	10	Nil
Meloidogyne spp.	Vegetable crops	15	Nil
	Fruit crops	12	2
	Culture pots	-	2
	Kitchen garden	-	1
Pratylenchus zeae	Grapes	1	1
	Gujarat	1	1
Rotylenchulus reniformis	Vegetable and pulse crops	5	Nil
Tylenchulus semipenetrans	Citrus	7	Nil

Fifteen samples of root-knot infested vegetable crops did not reveal *P. penetrans* incidence, however, among 12 samples collected from fruit crops, two (from grapes) harboured bacterium. *Pasteuria penetrans* infection was also recorded in the root-knot infected greenhouse cultures and a kitchen garden. Incidence of *Pasteuria* was also observed on *P. zeae* in two samples, one collected from grapes and the other in a population from Gujarat (Table 1).

It is apparent from the random survey that detectable levels of *Pasteuria* spp. are more likely to occur on perennial crops and under conditions that provide continuous and prolonged nemic build up such as greenhouse cultures.

Specific surveys of root-knot infested grape vineyards

Out of 11 vineyards, analysis of soil samples revealed the incidence of P. penetrans in four. However, the endospore encumberance per juvenile (1-5 endospores/ J2) and proportion of infected juveniles (10-90%) varied among vineyards (Table 2).

The high incidence of *P. penetrans* in root-knot infested grape vineyards could be due to introduction of nematodes along with its hyperparasite, *P. penetrans* through the rooted cuttings, and perennial nature of the host crop. In an annual cropping system, a susceptible host plant may not be available to the nematode (and also the bacterium) throughout the year. Furthermore, the agronomic practices may also disperse and "dilute" the bacterial population in soil reducing it to non-detectable levels. In a perennial cropping system, the host plant is available continuously, thus allowing the nematode as well as the bacterium to build up their populations in an undisturbed ecosystem.

A detailed study encompassing suitability of extraction method for *P. penetrans* encumbered J_2 and correlation of bacterial incidence with age of the vines was conduced during 2000-01. A total of 25 vineyards were surveyed in the villages Arya Nagar, Dobhi, Kharia and Kirtan (Dist. Hisar, Haryana) and the results are presented in Table 3.

Table 2. Incidence of *P. penetrans* in grape vineyards infested with root-knot nematode in District Hisar (1999-2000)

Village	No. J_2/200 cc soil	Percent J_2 of encumbered with endospores	No. of endospores /J_2
Dobhi-I	572	10.0	1-2
Dobhi-II	780	90.0	1-5
Kharia	2400	10.0	1-2
Salemgarh	10	0.0	-
Siswal-I	192	0.0	-
Siswal-II	450	0.0	-
Siswal-III	450	0.0	-
Siswal-IV	630	0.0	-
Siswal-V	120	0.0	-
Siswal-VI	300	0.0	-
Siswal-VII	140	20.0	1-2

Table 3. Natural incidence of *Pasteuria penetrans* in grape vineyards infested with root-knot nematodes in District Hisar (2000-01)

Village / Site no.	Ca. age of vines (yrs)	No. of J_2/200 cc soil		Per cent J_2 encumbered with endopsores		No. of endospores/J_2	
Arya Nagar		MBFT	SCFT	MBFT	SCFT	MBFT	SCFT
1	5	270	307	0.0	0.0	0.0	0.0
2	5	333	1008	0.0	0.0	0.0	0.0
3	5	216	1350	10.0	0.0	0.1	0.0
4	5	234	480	10.0	0.0	0.1	0.0
5	5	88	336	0.0	10.0	0.0	0.2
6	10	155	315	10.0	10.0	0.1	0.1
7	10	360	336	0.0	30.0	0.0	1.9
8	10	211	944	0.0	0.0	0.0	0.0
9	10	96	229	10.0	0.0	0.2	0.0
10	10	144	221	0.0	0.0	0.0	0.0
11	10	14	61	0.0	30.0	0.0	10.4
12	10	60	377	0.0	0.0	0.0	0.0
13	15	112	212	10.0	10.0	0.4	0.3
14	15	80	96	0.0	0.0	0.0	0.0
15	15	193	480	0.0	0.0	0.0	0.0
16	20	474	756	20.0	0.0	0.2	0.0
Dobhi							
17	10	333	400	0.0	0.0	0.0	0.0
18	10	252	252	40.0	20.0	0.7	0.4
19	10	63	156	0.0	0.0	0.0	0.0
20	10	243	760	10.0	20.0	0.1	0.3
21	10	312	422	10.0	10.0	0.1	0.1
Kharia							
22	10	450	675	20.0	0.0	0.4	0.0
23	15	106	300	0.0	0.0	0.0	0.0
Kirtan							
24	5	13	494	0.0	0.0	0.0	0.0
25	20	88	244	0.0	10.0	0.0	0.1

MBFT = Modified Baermann's Funnel Technique; SCFT = Sugar Centrifugal Floatation Technique

Suitability of extraction method for bacterium-infected nematodes

Root-knot nematode (*M. javanica*) was prevalent in all the 25 vineyards. The recovery of J2, in majority of cases, was higher by SCFT compared to MBFT (Table 3). Comparing the means of all the 25 samples, the average number of J2 recovered by SCFT was 448.4 and it was ca. 2.5x more than those recovered by MBFT (196). This can be attributed to the dependence of MBFT on the mobility of nematodes to pass through the tissue papers.

P. penetrans was recorded in 10 samples by MBFT and per cent J2 encumbrance ranged between 10-40; whereas by SCFT the incidence of bacterium was found in 9 samples and spore encumbrance varied from 10 to 30 per cent. Irrespective of MBFT or SCFT, the incidence of *P. penetrans* was observed in 14 samples (56 %). Overall average of all the 25 samples revealed that the per cent J_2 encumbered with *P. penetrans* endospores was the same, namely, 6 per cent both by MBFT and SCFT. Regarding average number of endospores per J_2, it varied from 0.1 to 0.7 by MBFT and 0.1 to 10.4 by SCFT. Considering all the 25 samples, the figures averaged 0.096 by MBFT and 0.552 by SCFT.

It has been reported earlier (Davies *et al.*, 1991) that nematodes heavily encumbered with *P. penetrans* endospores are less active and, therefore, may not be able to pass through the tissue paper. While counting the endospore encumbrance, occasionally a few juveniles having much higher endospore load by SCFT (e.g., site # 11, Table 3) were recovered. Obviously, such heavily encumbered juveniles failed to pass through the tissue papers and could not be recovered by MBFT. It is, therefore, suggested to use SCFT for working with *P. penetrans* in particular.

Age of vines v/s Incidence of *P. penetrans*

Further elucidation of data according to age of grapevines provided some interesting information. The age of the plantations varied from approximately 5 to 20 years. *M. Javanica* J2 population was much higher (1112 per 200cc soil) in younger vines (5-year-old) compared to vines more than 10-year-old (272-500 J2 per 200 cc soil), taking into consideration recovery by SCFT only. This could be due to exponential growth of the nematode at a new site immediately after its introduction. After attaining peak populations as observed on 5-year-old vines, a declining trend was observed in the older vines which stabilized nematode populations (maintenance level) at around 250-500 J2 per 200 cc soil.

The incidence of *P. penetrans* was found to be very low (1.7 per cent) in 5-year-old vines, highest (9.2 per cent) in 10-year-old vines, whereas in 15 and 20-year-old vines it was recorded as 2.5 and 5 per cent, respectively. Similar trend was observed regarding number of endospores per J2. It was least (0.03) in 5-year-old vines and maximum (1.0) in 10-year-old vines (Table 3).

P. penetrans is a hyperparasite of *M. javanica* and its build up depends upon the growth of its nematode host population. Logically, the growth of parasite (*P. penetrans*) followed the host (*M. javanica*). The results indicated that perhaps *P. penetrans* plays an important role in the natural regulation of *M. javanica* populations. After the nematode attained exponential growth on the younger vines it seems that natural biocontrol agents, including *P. penetrans*, limited the nematode population to maintenance levels when vines are about 15 to 20-year-old. With the decline of nematode population the decrease in the prevalence of *P. penetrans* is expected considering its obligate parasitic nature. Further detailed studies on the population dynamics of *M. javanica vis-à-vis P. penetrans* are desired to elucidate this phenomenon.

In a similar study conducted by Mani *et al.* (1999), the occurrence and distribution of *P. penetrans* in root-knot infested grape vineyards in Tamil Nadu was found to be mostly confined to vineyards of about 10-year-old. The root-knot nematode and *P. penetrans* seemed to maintain a balance in vineyards that were over 10-year-old. Stirling and White (1982) also reported the natural suppression of root-knot nematode by *P. penetrans* in older vines in Australia.

Bioassay

All the 14 samples, in which incidence of *P. penetrans* was recorded, whether by MBFT or SCFT, were further analyzed through bioassay conducted in the laboratory.

The bioassay confirmed the incidence of *P. penetrans* in all the 14 samples and it varied from 3.3 to 33.3 per cent and the mean number of endospores per J2 from 0.03 to 0.9. Lowest incidence was recorded at site no.6 and highest at site no. 20 (Table 4).

The results obtained on the incidence of *P. penetrans* during survey are not comparable for different sites because of the differences in the nematode populations and other edaphic factors. In the bioassay, the soil was air-dried to kill the resident nematode population while retaining the viability of the native *P. penetrans* endospores. Bioassay ensured uniform conditions in respect of *M. javanica* populations, age of juveniles, time of exposure, besides uniformity in the soil moisture and temperature conditions. Hence the results of bioassay appeared to be more authentic and revealed higher values for the average number of endospores per J_2 compared to those obtained in survey.

Analysis of data on bioassay presented in Table 4 shows non-significant differences on both the parameters among various sites, despite the fact that average number of endospores per J_2 varied from 0.03 to 0.9. This could be due to replication error. Chen and Dickson (1997) suggested the use of tally thresholds (T) to overcome this difficulty. Although this method requires a large sample size (25 to 50 J_2), it is relatively easy and rapid to determine a J_2 with $\leq T$ or $\geq T$ endospores attached. Thus, by choosing an appropriate T value, the number of endospores attached per J_2 can be determined easily and precisely.

Table 4. Natural incidence of *Pasteuria penetrans* in grape vineyards infested with root-knot nematodes in District Hisar – Estimation through bioassay

Site no.	Per cent J2 encumbered with Pp	Av. No. of endospores/J2
3	10.0 (19.44)*	0.23 (1.23)**
4	13.3 (22.39)	0.16 (1.16)
5	16.7 (25.12)	0.36 (1.36)
6	03.3 (11.47)	0.03 (1.03)
7	10.0 (19.44)	0.10 (1.10)
9	16.7 (25.12)	0.73 (1.73)
11	13.3 (22.39)	0.36 (1.36)
13	10.0 (19.44)	0.30 (1.30)
16	16.7 (25.12)	0.23 (1.23)
18	13.3 (22.39)	0.16 (1.16)
20	33.3 (36.24)	0.90 (1.90)
21	16.7 (25.12)	0.63 (1.63)
22	06.6 (16.00)	0.30 (1.30)
25	10.0 (19.44)	0.13 (1.13)
CDP=0.05)	(NS)	(NS)

* Angular transformed values; ** n+1 values; Pp = *Pasteuria penetrans*

Site # correspond to Table 3, known to be infested with Pp

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